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APR -7 1952

VOLUME 10 NUMBER 4

NUTRITION REVIEWS

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APR 10 1952
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April 1952

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

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Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.

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NUTRITION REVIEWS

VOL. 10 APRIL 1952 No. 4

CALCIUM IN THE CHEMISTRY OF FOOD AND NUTRITION

In 1894, Congress first showed recognition of nutrition and food supply as a nation-wide governmental concern by including an item of $10,000 (later $15,000) in the budget of the U. S. Department of Agriculture for investigations in human nutrition. Dr. W. O. Atwater, professor of chemistry in Wesleyan University (at Middletown, Conn.), was appointed special agent in charge of these nutrition investigations. The general plan was to determine, with as much quantitative precision as possible, the needs of human nutrition and the values of foods in meeting these needs. Under Atwater's initiative and supervision, about half of this appropriation was at once devoted to unprecedentedly precise experimental studies of the energy requirements of human nutrition, and the remainder to the composition and nutritive values of individual foods and of typical American dietaries.

In 1898, the writer was appointed as an "assistant in nutrition investigations" in the U. S. Department of Agriculture and had the good fortune to be assigned as a member of Professor Atwater's immediate staff. In addition to general assistance to Atwater, he was also given direct charge of work on the problem: which mineral elements, and under what conditions, require special attention that adequate amounts be furnished by ordinary American dietaries? Research groups in other laboratories were beginning to emphasize studies of mineral elements in nutrition at about this same time. Thus Moore wrote that proteins in isolation are inert, lifeless, bodies: "What puts life into them is the presence of electrolytes." Meltzer, from a pharmacologic viewpoint, wrote in 1908 that calcium is capable of correcting many disturbances of equilibrium in the body, whatever the directions of the deviations from the normal may be. He held that "any abnormal effect which sodium, potassium, or magnesium may produce . . . calcium is capable of re-establishing the normal equilibrium." A few years later, McCollum emphasized the fact that the phosphorus of plants tends to be concentrated in the seeds and their calcium in the leaves, and that in consequence dietaries consisting largely of seeds and their products need to be balanced by larger use of the calcium-rich foods, leaves, and milk with its products other than butter. Findings to this effect overlapped in time in McCollum's laboratory and that of the writer.

Among the food supply problems of the first world war and its aftermath was how much "protective" food must be contained in the diet in order to make it adequate, aside from the need for vitamin C.

In experiments with rats, covering entire lifetimes and often successive generations, it was found that a mixture of five-sixths ground whole wheat and one-sixth dried whole milk (which for convenience we here call Diet A) is *adequate*; but that, with the proportion of milk increased to one-third of the mixture (Diet B), the results are *better*. Growth and development are expedited, adult vitality is maintained at a higher level for a longer time, and the lives of normal adults are 10 per cent longer; corresponding to an extension of the human adult *life expectation*, from the long-standing 70, to 77 years.

The increased proportion of milk increased the intakes of at least four nutrients, of which the most influential, in experiments of this type, have been found both in the Johns Hopkins and the Columbia University laboratories, to be calcium and vitamin A.

Normally the body so uses its supplies as to keep the calcium content (or, more strictly speaking, the concentration of cal-

cium ions) in the blood very nearly constant. This relative constancy of the blood calcium is chiefly a direct effect: (1) of the physicochemical properties of the calcium-protein compounds; and (2) of the bone trabeculae. These trabeculae are crystals of calcium compounds, chemically similar to those of bone, which grow from the inner surface of the cavity at the end of the bone, thus increasing the area of bone-salt material with which the blood comes in contact as it circulates through the vascular ends of the bones, and thus tending quickly to restore any depletion of calcium which the blood may have suffered in its circulation through the body, and its functioning to maintain the near-constancy of the blood-calcium content.

Many experiments were then performed in which the above-described Diet A was enriched step-wise in calcium while the concentrations of the other nutrients remained unchanged. These experiments showed that the superiority of Diet B to Diet A was largely due to its increased calcium content.

Bauer, Aub, and Albright (1929) and also W. B. Cannon (1939) have explained how, in the body which has been well supplied with food calcium, the bone trabeculae serve as a calcium reserve, readily drawn upon whenever there is, or tends to be, a loss of calcium from the blood and soft tissues.

The physicochemical behavior of calcium in the blood, the calcium balance experiments in human beings of all ages, and analytic determinations of calcium in laboratory animals as affected by age and by food—all drew increasing attention to calcium as a factor in human nutrition.

Both from the viewpoints of biochemical theory and of health significance, much importance attaches to the fundamental question of the amounts of calcium needed for the maintenance of normal adult equilibrium as well as for the requirements of optimal growth, reproduction, and lactation.

Up to about thirty years ago the literature of calcium balance experiments in man showed 97 cases in which, according to the state of knowledge at that time, the calcium output might be tentatively regarded as throwing light upon the maintenance needs of normal men and women. These daily outputs averaged 0.45 g. per 70 kg. of body weight per day. Because of individual variations and uncertainties of interpretation, it was suggested that this figure be increased by 50 per cent to obtain a tentative standard allowance for use in practical dietetics.

By 1937, further data had been published and all the data then available which were deemed sufficiently valid were collated and interpreted in a new way by Leitch (1937, 1938). Her average finding for adult maintenance was 0.55 g. of calcium for normal human adults regardless of weight and sex. In older men than those included in the previous compilations, Owen found an average requirement of 0.52 g. of calcium a day.

The first Recommended Allowances of the National Research Council, published in 1941, provided 0.8 g. of calcium a day for the normal maintenance of either man or woman, regardless of muscular activity. In discussion, the allowance of as much calcium for women as for men, notwithstanding the higher body weight of the latter, was explained as a "free-hand" recognition of the desirability of generosity of calcium intake in anticipation of the extra demands of pregnancy and lactation on a considerable fraction of the females of the population.

Independently Obermer concluded from his study of 80 cases of pregnancy that the minimum safety level for daily calcium intake is not less than 2.0 g. in the first to seventh months of pregnancy, and 2.5 g. from the eighth month to term.

There is also other clinical evidence favorable to liberal calcium intake. Dr. F. J. Stare, speaking at the 1943 Conference on Nutrition in Relation to Public Health, said of his hospital experience: "It was a

surprise to me to see that the majority of x-ray studies of adults past the age of 45 to 50 years showed considerable demineralization of bone, and one wondered if low dietary intake of calcium, or of vitamin D, over many years, might not have been prominent factors in the etiology of this demineralization." A marked frequency of finding of calcium-poor condition of body in middle-aged people, who had been supposed to be normal, has been informally reported from the middle west.

Two series of over 100 cases each have been studied by a method of measured but not controlled intakes, and statistically interpreted by "regression of balance upon intake." The large volume of data thus obtained by a somewhat different method consistently points to 1.0 g. of calcium a day as a desirable allowance.

During 1941 to 1947 the attention of the National Research Council (U. S.) was so concentrated upon vitamin problems that calcium requirement remained without critical attention. When such critical study was resumed, it became apparent that the compilation of 1920 in seeking comprehensiveness had been unduly receptive to data in which calcium output exceeded calcium intake, with the result that the tentative *mean* was seen to be too low, while also individual variability of calcium requirement was found to have been very inadequately appreciated.

During 1947–1948 the Committee on Dietary Allowances of the National Research Council re-examined and, where necessary, resifted the data, with an outcome of 73 cases now apparently eligible for use in drawing a new mean. This new recommendation is 1.0 g. of calcium per normal adult per day. These 73 cases represent various parts of the world, and statistically the average of 1.0 g. meets the requirements of all but about one person in each 100 of the population.

At first thought, some critics incline to the view that it may be carrying overgenerous insurance against calcium deficiency when we set an allowance which is sufficient to cover the need of all but one in 100 of the normal population. But a second thought is importantly reassuring. It is that a generous surplus in our daily food is *not only* insurance against deficiency. It also makes a real contribution to our positive health, for it has now been shown by hundreds of experiments with animals whose nutritional chemistry is much like our own that starting with a diet adequate in all respects we can make it more efficient by increasing its calcium content.

On carrying such experiments through entire lives and successive generations, it becomes both clear and conclusive that wherever, within normal bounds, we have a choice of higher or lower calcium intake levels, the choice of the more liberal consumption of calcium may confidently be expected to contribute more both to immediate efficiency and to the higher health of the long run of the life cycle.

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INDIVIDUAL ESSENTIAL AMINO ACIDS IN PLASMA AND URINE OF SURGICAL PATIENTS

There have been many studies attesting to the importance of nutrition in surgical patients (*Nutrition Reviews* **3,** *40, 86, 253 (1945)*; **4,** *6 (1946)*; **5,** *46 (1947)*; **6,** *20, 161 (1948)*; **7,** *123 (1949)*; **8,** *137 (1950)*; **9,** *257 (1951)*). Derangements of protein metabolism in the acutely or chronically ill have been particularly emphasized. Impaired healing of wounds, increased susceptibility to anesthesia, shock and infection, mal-

functioning of the liver and intestinal tract (including operative anastomoses) are some of the complications observed in protein-deficient patients. Under these circumstances, operative deaths are more frequent, convalescence is prolonged, and mortality increased.

One of the problems in the care of seriously sick patients is the difficulty of accurately assessing nutritional status. Appraisal of probable dietary intake, body weight and height, blood volume (red cell and plasma), and circulating hemoglobin are all suggestive indications of the state of protein nutrition. The usefulness, but limitations, of measurements of plasma protein (concentrations, fractions, and total circulating) have been described—the plasma protein may reflect, but tardily, the state of tissue proteins.

E. B. Man, P. G. Bettcher, C. M. Cameron, and J. P. Peters (*J. Clin. Invest.* **25,** *701 (1946)*; *Nutrition Reviews* **5,** *329 (1947)*) found lower than normal plasma concentrations of total alpha-amino nitrogen preoperatively in some malnourished patients. This finding suggested to T. C. Everson and M. J. Fritschel (*Surgery* **30,** *931 (1951)*) that measurements of the plasma levels of the essential amino acids rather than total amino acid nitrogen might yield particularly useful information regarding nutritional status. Accordingly, they have determined by microbiologic technics the fasting plasma levels of the eight individual amino acids (free) essential for man (leucine, isoleucine, tryptophan, valine, methionine, threonine, phenylalanine, and lysine) and of histidine and arginine in 63 surgical patients having various pathologic conditions (specific diagnoses not tabulated). Thirty-eight of the patients were considered to be in poor nutritional status on the basis of one or more of these criteria: (1) loss of 10 per cent or more of previously normal body weight; (2) clinical appearance of emaciation; (3) serum albumin level below 4.6 g. per 100 cc. of serum. The other 25 were considered to be in good nutritional status by these same criteria. It is stated that the two groups were made up of patients having approximately the same pathologic conditions.

There was no statistically significant difference between the mean total of the ten amino acids found in the previously well nourished patients (174.1 micrograms per cubic centimeter of plasma) and that reported previously by S. W. Hier (Thesis: "Studies on the Free Amino Acids of Blood and Sweat," *University of Illinois (1947)*) in healthy medical students (181.3 micrograms per cubic centimeter of plasma). The mean total of the poorly nourished group (131.4 micrograms per cubic centimeter of plasma), however, was significantly lower than both of the above groups. Similarly, the mean value of each of the individual amino acids measured (except arginine) was significantly lower in the malnourished group. The reduction was about 45 per cent for isoleucine and methionine, 25 to 35 per cent for threonine, phenylalanine, tryptophan, leucine, lysine and histidine, 17 per cent for valine, and 8 per cent for arginine.

Eighty-five of the 380 determinations of the plasma levels of the various individual amino acids in the group of malnourished surgical patients were more than two standard deviations below the corresponding mean plasma levels in the well nourished patients. A larger number, 133, were more than two standard deviations below the corresponding mean plasma levels in normal students. Isoleucine, histidine, and lysine were particularly low. In over 50 per cent of the poorly nourished group, the plasma levels of these three amino acids were lower by more than two standard deviations than the corresponding mean levels in normals. This presumably would explain, in large part, the similar finding regarding the total values for the ten amino acids, since these three amino acids comprise such a large proportion of the ten measured.

Among the 38 malnourished patients no significant correlation was observed between

the average total values of the ten (free) amino acids and the presence of carcinoma (18 patients), clinical emaciation (21 patients), hypoalbuminemia (13 patients), or impaired liver function (8 patients) considered singly. No attempt was made to correlate the plasma data with the simultaneous presence of two or more of these conditions. Regarding the individual amino acids, in only a few instances were statistically significant differences observed in these various subgroups. Leucine and isoleucine were significantly lower in the patients without carcinoma, valine in the emaciated and hypoalbuminemic groups, and valine and isoleucine in those with impaired liver function.

From the data presented, Everson and Fritschel suggest that decreases in plasma concentrations of the total level (free) of the ten amino acids studied, or of isoleucine, histidine, or lysine, may indicate protein malnutrition. Since these decreases may be observed at a time when the plasma albumin concentration is still normal, they suggest that a decrease of the essential amino acids in plasma may be a useful early biochemical sign of protein malnutrition. One may anticipate as much difficulty in the interpretation of plasma amino acid concentrations as in that of other plasma constituents. Thus, though it would appear that in a certain percentage of malnourished patients lowered plasma levels of some of the essential amino acids may be found, does this finding mean significant tissue protein depletion? The plasma amino acid level may, for example, reflect only a diminished (or abnormally balanced) intake in recent days. J. B. Kirsner, A. L. Sheffner, and W. L. Palmer (*J. Clin. Invest.* **28,** *716 (1949)*) found in one human subject that the oral administration of a peptone solution treated with hydrogen peroxide and containing markedly reduced quantities of methionine, lysine, histidine, leucine, isoleucine, valine and threonine, was accompanied by definite decreases in the free levels of these amino acids in the plasma. During the same periods, the amino acid outputs in the urine increased considerably. These findings point to the importance of considering recent dietary intake and urine output in evaluating plasma concentrations. Some aspects of this have been previously reviewed (*Nutrition Reviews* **5,** *43, 213 (1947)*; **6,** *53 (1948)*; **7,** *44, 115 (1949)*; **8,** *215 (1950)*). Data are presented briefly by Everson and Fritschel on 5 malnourished patients which indicate that the decreased levels of the free amino acids measured by them are not restored to normal by only a few days of high protein (level not stated) diets.

The plasma amino acids are apparently in dynamic equilibrium with tissue amino acids and proteins. It is to be anticipated that changes in the tissues would be reflected, directly or indirectly, in changes in the plasma amino acids. F. L. Engel, M. G. Winton, and C. N. H. Long (*J. Exp. Med.* **77,** *397 (1943)*; *Nutrition Reviews* **1,** *364 (1943)*) observed a rise in plasma alpha-amino nitrogen in hemorrhagic shock in rats; a concomitant fall in red cell alpha-amino nitrogen occurred.

Christensen and his co-workers (*Nutrition Reviews* **7,** *203 (1949)*) have shown that high plasma levels of some amino acids (*e.g.*, proline) brought about by feeding appeared to interfere, perhaps on a competitive basis, with the cellular activity for concentrating other amino acids. When glutamic acid was fed in excess, however, the concentrating activity of liver and muscle for certain amino acids appeared to be increased and there was a concomitant fall in the plasma levels of these amino acids. From this point of view, decreased plasma levels of amino acids may result from increased concentrative assimilation by the cells. Elevated levels may occur when there is inhibition of the concentration process. In a similar vein C. H. Li, I. Geschwind, and H. M. Evans (*J. Biol. Chem.* **177,** *91 (1949)*), have demonstrated that the administration of anterior pituitary growth hormone causes

a significant decrease in the blood amino acid content in rats. Li and co-workers found that in normal rats the effect occurs within six hours and disappears after twelve hours. One milligram of the hormone seemed to be an optimal dosage for maximal lowering. They interpret this as a reflection of the protein anabolic properties of the growth hormone. In a similar fashion, they interpret an observed increase in plasma alpha-amino nitrogen following the administration of adrenocorticotropic hormone (5.0 mg. of the hormone given intraperitoneally in divided doses daily for four days) as a reflection of its growth-inhibiting properties.

The plasma levels of amino acids may be influenced by various pathologic conditions. Elevations are seen in conditions characterized by severe liver or renal dysfunction. An increase has also been observed in rats consequent to hemorrhagic shock (Engel, Winton, and Long, *J. Exp. Med., loc. cit.*) and following severe burns in rats (H. N. Harkins and Long, *Am. J. Physiol.* **144,** *661 (1945)*), calves (W. W. L. Glenn, J. Muus, and C. K. Drinker, *J. Clin. Invest.* **22,** *451 (1943)*) and humans (S. M. Levenson *et al.*, *New Engl. J. Med.* **235,** *467 (1946)*). Decreases in plasma total alpha-amino nitrogen concentration have been reported during certain infectious diseases (L. E. Farr, W. C. McCarthy, and T. Francis, *Am. J. Med. Sci.* **203,** *668 (1942)*) and nephrotic crises (Farr and D. A. MacFadyen, *Am. J. Dis. Child.* **59,** *782 (1940)*). No attempt was made to correlate the plasma amino acid levels with nutritional status. As already noted, Man *et al.* found decreases of plasma alpha-amino nitrogen in some malnourished preoperative surgical patients. Postoperatively there was a transient fall in those patients with normal, or near normal, levels preoperatively. The degree and duration of the decline appeared related to the severity of the operative procedure. Much less change was seen postoperatively in the malnourished patients with low levels preoperatively.

In a subsequent study (E. B. Man, P. G. Bettcher, and W. T. Brown, *Yale J. Biol. Med.* **20,** *167 (1947)*), it was found that the plasma alpha-amino nitrogen concentrations were below the average of normals in 31 of 38 schizophrenic patients. Insufficient data are presented to attempt a correlation of the observed levels and nutritional status. In 7 patients studied over a period of time the plasma amino acids fell in acute exacerbations and rose when the patient improved symptomatically. The decreases were correlated with acute manifestations of the disease, not with decreased caloric intakes or overactivity. It was felt that the increases observed did not result from the metabolic effects of electroshock. Seven schizophrenic patients were subjected to prefrontal lobotomy (Man, Bettcher, C. M. Cameron, and Brown, *Ibid.* **20,** *175 (1947)*). Decreases in plasma alpha-amino nitrogen, serum cholesterol, fatty acids, proteins, and albumin in the first one or two days after operation occurred in the same manner as in nonschizophrenic patients after serious operations. Later in the postoperative course, the plasma alpha-amino nitrogen in the operated schizophrenic rose to levels seen in normals. The authors offered no interpretation of the observation that the rise in amino acids coincided with improvement in the schizophrenic state. It is not possible to determine from the data presented whether there was any definite correlation of the plasma amino acid levels with nutritional status.

A factor bearing on the interpretation of plasma concentration data is the size of the plasma compartment; "false-high" or "false-low" levels might be obtained consequent to certain fluid and electrolyte shifts not directly related to the state of protein nutrition. Everson and Fritschel feel that the differences in plasma amino acid levels observed by them in the malnourished patients as compared with the well nourished surgical patients were not on the basis of plasma dilution, at least as deduced from a comparison of hematocrit levels. The mean hemato-

crit level for the well nourished patients was 43.5 per cent, for the malnourished, 41 per cent. The uncertainty of estimating plasma volume from hematocrit measurements is recognized by the authors. Measurements of the various body fluid compartments would be useful in this regard.

Everson and Fritschel measured twenty-four-hour urinary excretion of free and total individual essential amino acids in 5 malnourished surgical patients on ward diets. There were no definite variations in the pattern of amino acid excretion by these patients from the published values for normal individuals on average diets. These studies were not extended, however, because of dissatisfaction by these investigators with the degree of reproducibility of determinations of the free amino acids in urine samples.

The study carried out by Everson and Fritschel is a stimulating one. Extension is certain to yield useful information regarding protein metabolism and nutrition. They call attention to the considerable differences in the amount and percentage of decrease of the various individual amino acids studied by them. They suggest the need for investigation of the possibility of accelerating protein synthesis in malnourished surgical patients by reinforcing the diet with those amino acids which showed the greatest decrease, especially methionine and leucine.

INCREASING THE ASCORBIC ACID CONTENT OF VEGETABLES AND FRUITS

Work is progressing in several laboratories on the problem of increasing the content of ascorbic acid in vegetables and fruits. These are very worthwhile enterprises, since vitamin C is a major constituent of vegetables from the point of view of human nutrition. Any improvement that can be made in the inherent ability of our common varieties to synthesize and store ascorbic acid is a permanent contribution to our food supply.

Tomatoes have been the subject of special efforts by R. E. Lincoln, G. W. Kohler, W. Silver, and J. W. Porter (*Bot. Gaz.* **111,** *343 (1949–50)*) at Purdue University. A survey of the genus *Lycopersicon* demonstrated a wide range of vitamin C contents, from 100 to 1050 parts per million (p.p.m.), the highest values being found in *L. peruvianum* and *L. pimpinellifolium*, both small-fruited species. From this field-grown material, selections were made for the breeding program, which had several objectives. A better understanding of the inheritance mechanism was desirable, and the relation of vitamin C content to fruit size and other characters should be known to permit more intelligent plant breeding work designed to combine high vitamin content with other desirable characteristics in the same variety.

Several segregating populations derived from crosses between small- and large-fruited strains with high and low ascorbic acid contents, respectively, showed a highly significant negative correlation between ascorbic acid content and fruit size. A selection experiment through 6 generations of a segregating population demonstrated that many genes are concerned in the production of high ascorbic acid content and that environmental factors greatly affect this characteristic (*Nutrition Reviews* **7,** *173 (1949)*). A backcrossing program was started, using 2 varieties, Rutgers and Indiana Baltimore, as the recurrent parent for fruit size. Twenty small-fruited accessions with high vitamin C contents were used in the first crosses. Out of 1600 progeny in 1947, one parent was responsible for the high ascorbic acid content of 80 per cent of the best 150 selections. Although fruit sizes did not quite equal those of the recurrent parents, the ascorbic acid content was twice that of the commercial parents. Fruit sizes were 5 ounces (140 g.) and contained 500 p.p.m. ascorbic acid. This is comparable to the

vitamin C content of citrus fruits. The recommended daily allowance for an active man would be contained in 135 g. of tomato. These results are very encouraging, and further work is in progress.

The influence of nitrate supply upon the ascorbic acid content of tomatoes was reported by G. F. Somers, W. C. Kelly, and K. C. Hamner (*Am. J. Bot.* **38,** *472 (1951)*). Tomatoes were grown in sand culture with varying supplies of nitrate. High nitrate supply for the month prior to the ripening of fruit was associated with high fruit production, but with lower ascorbic acid content by about 10 per cent. Nitrate supplied after ripening had started did not affect either yield or ascorbic acid content. At the last harvest, ascorbic acid content was associated with the degree to which the fruits were shaded. Fruits in full shade contained about 12 per cent less ascorbic acid than those fully exposed to sunlight. Differences in vegetative growth associated with variations in nitrate supply prior to ripening may result in shade differences which are responsible for the observed ascorbic acid differences. These seem too small to be of any practical nutritional significance.

A survey of strawberry varieties was made in Mississippi by J. P. Overcash and L. McWhirter (*Proc. Am. Soc. Hort. Sci.* **52,** *251 (1948)*) in an effort to find parents suitable for improving the vitamin C content for dietary purposes. Nine varieties were studied. Replicates were treated statistically over a three-week period, analyses being made at weekly intervals. On a fresh weight basis, the seasonal average ascorbic acid content ranged from 43 to 67 mg. per 100 g. A significant decrease of 25 per cent on a moisture-free basis occurred during the season. This range among varieties does not appear encouraging for improvement by plant breeding. Perhaps better germ plasm might be found among wild species of strawberries.

VITAMIN B_{12} DEFICIENCY IN SWINE

Since the hematologic, neurologic and gastrointestinal manifestations of Addisonian pernicious anemia in human subjects respond so dramatically to the parenteral administration of small doses of vitamin B_{12}, it might be expected that a deficiency of vitamin B_{12} in animals would be associated with manifestations such as those seen in pernicious anemia, *i.e.*, macrocytic anemia, leukopenia, morphologic alterations in the leukocytes, thrombocytopenia, megaloblastic bone marrow, an increase in serum bilirubin and bile pigment excretion, subacute combined degeneration of the nervous system, and glossitis. In order to determine this G. E. Cartwright and co-workers (*Blood* **6,** *867 (1951)*) fed a total of 70 weanling pigs various vegetable protein diets deficient in vitamin B_{12} and made careful studies on the blood and bone marrow.

Two different experiments were performed. In the first experiment 43 baby pigs, 2 to 7 days of age, were fed a soybean alpha-protein, "synthetic milk" diet with a methionine supplement. Folacin was included in the diet. Seven pigs served as controls and were given vitamin B_{12}. Desiccated thyroid was added to the diet of 20 of the pigs since it has been shown that the administration of this substance increases the vitamin B_{12} requirement. Only 21 of the animals survived for a sufficiently long period for adequate evaluation of the results.

Growth was considerably better in the control animals than in those animals not receiving vitamin B_{12}. Of the 17 deficient animals, no anemia was observed in 6, a mild anemia (volume of packed red cells 30 to 39 ml. per 100 ml.) was found in 10, and in only one animal was severe anemia present. Anemia when present was normocytic and not macrocytic. The bone marrow

revealed a slight increase in normoblasts, but no megaloblasts were present. Following therapy with crystalline vitamin B_{12} a reticulocytosis was observed, but there was only a slight increase in the volume of packed red cells.

In the second experiment 27 weanling pigs, 21 to 28 days of age, were fed a basal diet consisting of soybean alpha-protein, sucrose, lard and salt mix. Succinylsulfathiazole, vitamins A, D, E, K, and nine B vitamins excluding folic acid and vitamin B_{12} were given as supplements. The animals were divided into five groups. The first group was given a folacin and methionine supplement. The second group was given folacin, and the third group received neither folacin nor methionine. The animals in the fourth group were gastrectomized and received neither folacin nor methionine. Iodinated casein and folacin were added to the basal diet of the animals in the fifth group.

The addition of methionine to the diet enhanced growth considerably. The omission of folacin from the diet resulted in poor growth, as did gastrectomy and the addition of iodinated casein. Severe anemia was not observed in any of the pigs. A mild, normocytic anemia was present in the second, third and fourth groups. The sternal marrow differential counts in all groups revealed a slight increase in the proportion of erythroid cells, but no cells resembling megaloblasts were found. Leukopenia, morphologic alterations in the leukocytes, glossitis, and evidence of neurologic disease were lacking.

In view of the striking hemopoietic response following the administration of vitamin B_{12} in pernicious anemia, the failure to produce a megaloblastic macrocytic anemia in swine in which vitamin B_{12}-deficiency was produced is surprising. There are several possible reasons for this failure. The first is that the deficiency produced, though severe enough to impair growth, was not sufficiently pronounced to produce hematologic or neurologic alterations corresponding to those seen in pernicious anemia in human subjects. A second possibility is that a deficiency of vitamin B_{12} in the pig, in the absence of a folacin deficiency, may not be manifested by macrocytic anemia, since the hemopoietic disorder in the human disease can be corrected by the administration of folacin. If this is true, it would then mean that folacin is the vitamin more intimately concerned in maintaining the integrity of the hemopoietic system and that vitamin B_{12} is perhaps concerned in some more indirect manner involving the metabolism of folacin. This is also suggested by the observations made by G. E. Cartwright, B. Tatting, H. Ashenbrucker, and M. M. Wintrobe (*Blood* **4,** *301 (1949)*) that pigs made deficient in folacin develop a severe macrocytic anemia, leukopenia, thrombocytopenia, and morphologic alterations in the nucleated red cells in the bone marrow.

CORN TREATMENT AND PELLAGRA

Pellagra is generally associated with populations eating relatively large quantities of corn. This is true not because corn is necessarily deficient in niacin, for it compares favorably with other cereal grains in niacin content, but because the proteins in corn are deficient in tryptophan, the amino acid that animals are able to convert into niacin. The low tryptophan content, and possibly an imbalance in content of other amino acids in corn, can explain its pellagragenic action. It was proposed several years ago that corn is pellagragenic because of the presence of a toxic compound (*Nutrition Reviews* **5,** *163 (1947)*). This has, however, been questioned (*Ibid.* **6,** *77 (1948)*).

The effect of processing on the various fractions of the corn grain as it may influence tryptophan and niacin nutrition has recently received attention. J. Laguna and K. J.

Carpenter at the Rowett Research Institute in Scotland (*J. Nutrition* **45,** *21 (1951)*) have studied the influence of raw versus processed corn in niacin-deficient diets for the rat.

The following corn preparations were used in the study: lime-treated whole yellow corn; untreated whole yellow corn; reconstituted corn mixture from various fractions obtained in the "wet-milling" of corn; lime-treated corn starch; and a mixture of lime-treated corn germ, gluten and feed meal.

Weanling rats served as experimental animals and the experimental diets had the following percentage composition: alcohol-extracted casein, 7; L-cystine, 0.15; linseed oil, 2; cod liver oil, 1; a corn preparation (see above), 42; sucrose-mineral-vitamin mixture, 47.85. Each 58 g. of the sucrose-mineral-vitamin mixture contained the following vitamins (in milligrams): thiamine, pyridoxine, riboflavin, folacin, 2 methyl-1,4-naphthoquinone, 0.3 each; calcium pantothenate, 2; tocopherol acetate, 1.5; biotin, 0.01, and choline chloride, 200.

When the diet contained lime-treated corn, the rats gained 80 g. in body weight in four weeks. This was contrasted with a gain of only 40 g. when the diet contained raw corn. Supplementing the diets of either raw or lime-treated corn with 3 mg. of niacin per 100 g. of diet resulted in body weight gains of 70 to 75 g. in four weeks. Thus, lime treatment of the corn appeared to replace completely the supplementary niacin in the diet. The lime treatment consisted of heating 2500-gram batches of corn for forty to fifty minutes at 80°C. in 60 g. of calcium oxide and 6 l. of water. After standing overnight, this was neutralized with approximately 20 ml. of syrupy phosphoric acid and the whole mixture dried at 80° C. Since this final product contained approximately 2 parts in 40 of added tricalcium phosphate, all control corns likewise were prepared to contain 2 parts in 40 of added tricalcium phosphate.

The reconstituted fractions obtained from the commercial wet-milling process for corn were also compared with raw whole corn. This is a standard process used widely in the commercial preparation of corn products. Cleaned corn is steeped in dilute sulfurous acid, kept at 52° C. for thirty-six to forty-eight hours, and then separated into the various fractions (starch, gluten, germ, and feed meal).

The reconstituted mixture used consisted of the following: starch, 28; germ, 5; feed meal, 5.5; corn gluten, 1.5; and tricalcium phosphate, 2 parts.

The body weight gains of rats fed the reconstituted corn were only slightly greater than those obtained when raw whole corn was fed, and in both cases the growth rate was slow (15 to 20 g. in twenty-one days).

Supplementation with niacin also resulted in slightly better weight gains on the reconstituted corn diet than on the raw corn diet (50 and 40 g. respectively in twenty-one days). These results indicated that the fairly severe treatment given to commercially processed corn did not have the niacin-sparing effects that were obtained with lime treatment.

In order to determine which fraction of the corn was benefited by the lime treatment, a diet containing reconstituted corn with only corn starch lime-treated was compared with a similar diet in which the mixture of corn germ, feed meal and gluten was lime-treated. The diet containing the reconstituted corn with the lime-treated germ, meal and gluten produced nearly as good growth as did either diet supplemented with niacin (35 to 40 g. body weight gain in twenty-one days). The animals on the diet containing the reconstituted corn with only the starch fraction lime-treated made no better weight gain than the rats receiving reconstituted corn with no lime treatment.

Since lime treatment appeared to exert its niacin-sparing effects on the nonstarch fraction of corn, the authors considered their results were in harmony with the recent

findings by D. K. Chaudhuri and E. Kodicek (*Biochem. J.* **47,** *xxxiv (1950)*) and Kodicek (*Ibid.* **48,** *viii (1951)*). These workers noted that corn and other cereal grains contain precursors of niacin which are normally unavailable to the rat, and which are made available by alkaline but not by acid treatment.

The niacin-sparing effects of lime-treated corn, as reported by the British workers, are considered of particular interest because very little primary pellagra occurs in Mexico, yet the human consumption of corn is very high in many sections of that country. Since corn in Mexico is eaten primarily in the form of "tortillas," which involve cooking in lime water, the British results may offer an explanation for the low incidence of pellagra in this corn-eating population. Of incidental interest is the improvement in the calcium content of the diet that results from the use of lime-treatment of cereal products.

ANTIBIOTICS AND B-VITAMIN REQUIREMENTS

The growth-promoting effects of antibiotics in animals have been observed by many investigators. Many of the recent results have emphasized that the antibiotics have a particularly striking growth-stimulatory effect in crude diets that are deficient in vitamin B_{12} and also possibly in some growth factors which are still unknown.

That the effects of the antibiotics appear to be general has been emphasized by the recent results of workers at the University of Wisconsin. H. Linkswiler, C. A. Baumann, and E. E. Snell (*J. Nutrition* **43,** *565 (1951)*) reported that aureomycin increased the growth of rats that were fed a diet limited in vitamin B_6.

The Wisconsin laboratory has studied further the effects of several antibiotics on the growth of rats that were receiving limited amounts of thiamine, riboflavin, or pantothenic acid (Hwa Lih and C. A. Baumann, *J. Nutrition* **45,** *143 (1951)*). Groups of 5 weanling rats were fed diets deficient in one of these vitamins. Limited amounts of the vitamin with and without an antibiotic were included, as in a bioassay, after varying depletion periods. The influence of 3-nitro-4-hydroxyphenyl arsonic acid (50 mg. per kilogram of diet) and sulfasuxidine (1 per cent in the diet) were also tested under similar conditions. The antibiotics were usually fed at a level of 50 mg. per kilogram of diet.

The basal diet had the following percentage composition: sucrose, 73; vitamin-free casein, 18; salts, 4; and corn oil containing 0.002 per cent halibut liver oil and 0.003 per cent alpha-tocopherol, 5. In addition the diet contained the following vitamins (in milligrams per kilogram): pyridoxine, 2; niacin, 25; 2-methyl-1,4-naphthoquinone, 10; inositol, 100; biotin, 0.1; folic acid, 2; choline chloride, 1000; and vitamin B_{12}, 0.02. Thiamine, riboflavin and pantothenic acid were each included at varying levels. Adequate levels of these three vitamins were considered to be (in milligrams per kilogram of diet): thiamine, 2; riboflavin, 3; and calcium pantothenate, 20.

Rats receiving 0.5 mg. of thiamine per kilogram of diet and no antibiotic weighed 55 g. after a four-week feeding period, contrasted with weights of 69, 76, and 115 when the same diet was supplemented with streptomycin, aureomycin, or penicillin respectively. Essentially similar growth-stimulation from these antibiotics was observed when the diet contained thiamine at a level of 1.0 mg. per kilogram of diet. Chloromycetin and terramycin did not stimulate growth under these conditions. The response with sulfasuxidine appeared to be somewhat delayed. The growth-stimulatory effect of this drug was equal to that of aureomycin between the second and fourth weeks of the experiment.

Penicillin appeared to be as effective when

supplied in the diet at a level of 10 mg. per kilogram as when supplied at a level of 50 or 100 mg. Penicillin inactivated by penicillinase was inactive and 3-nitro-4-hydroxyphenyl arsonic acid was likewise inactive.

Aureomycin and penicillin both stimulated growth of rats receiving either 0.25 or 0.50 mg. of riboflavin per kilogram of diet. The amount of growth stimulation was about equal to that obtained by doubling the riboflavin in the diet. For example, rats on 1.0 mg. of riboflavin per kilogram of diet weighed 116 g. at five weeks compared to 118 g. in those receiving one-half this amount of riboflavin and 50 mg. of penicillin per kilogram of diet. Streptomycin, chloromycetin, terramycin and sulfasuxidine were only mildly active on marginal riboflavin intakes.

The growth-stimulating action of aureomycin or penicillin on marginal riboflavin intakes was paralleled by increased liver storage of this vitamin. Livers from rats on 0.5 mg. of riboflavin per kilogram of diet contained 51.7 micrograms of riboflavin, whereas the livers from rats on similar diets receiving penicillin contained 82 micrograms of riboflavin.

Rats receiving limited amounts of dietary pantothenic acid (0 to 4 mg. per kilogram of diet) grew better when the diet was fortified with aureomycin, streptomycin or penicillin than in the absence of these antibiotics.

These results are of particular interest in view of recent reports that the extensive use of antibiotics in human patients may lead to vitamin deficiencies (*Nutrition Reviews* **9,** *283 (1951)*). The presently reported experiments appear to be in conflict with these reports and again raise the question of the role of the antibiotics as they may influence intestinal bacteria in the synthesis and utilization of vitamins in humans. For example, it has been reported (*Nutrition Reviews, loc. cit.*) that aureomycin and chloramphenicol therapy in man results in a marked stimulation of yeast growth in the feces, and the feeding of live yeast has been demonstrated to increase the requirement for thiamine in man.

The results of the Wisconsin workers do, however, suggest that the growth-promoting action that has been repeatedly reported when antibiotics are added to crude diets may well be associated with marginal or submarginal intakes of known B-vitamins. Unfortunately, their studies included only limited data on the influence of the antibiotics when all known B-vitamins were fed at adequate levels. In their results with pantothenic acid, rats made somewhat better weight gains when the diet contained aureomycin plus presumably adequate quantities of pantothenic acid (48 mg. per kilogram of diet) than when the aureomycin was omitted. At five weeks those receiving aureomycin averaged 242 g. in body weight, compared with an average of 229 g. in the absence of the antibiotic. In the riboflavin and thiamine studies, no data were presented to indicate the influence of the antibiotics in the presence of adequate amounts of these vitamins.

More work is needed before any sound conclusions can be drawn concerning the mode of action of the antibiotics in influencing the requirements for known dietary factors. It is of course only speculation that an antibiotic apparently can conserve a given vitamin for the host by suppressing the intestinal organisms that are in competition with the host for that vitamin in the intestinal contents. This is the explanation offered by the Wisconsin workers for their results.

It can also be speculated that the antibiotics render their effects by improving the efficiency of utilization of all nutrients available to the host. Aureomycin residues still display growth-promoting activity after an alkaline treatment sufficient to destroy their antibacterial activity for *Staphylococcus aureus* (*Nutrition Reviews* **8,** *298 (1950)*).

Evidence has also been reported that certain of the sulpha drugs have a direct action on tissues which protects and spares the thiamine requirement in human beings and in rats (*Nutrition Reviews* **9,** *10 (1951)*).

MANAGEMENT OF OVERWEIGHT

In years past it has been principally the fair sex that supplied willing subjects for a long series of uncontrolled experiments on a variety of methods for weight reduction. Times have changed. At present the men have joined the women and are now eager to listen to a lecture or a sales talk on how to reduce. Their motives have also changed. It is often a concern for preservation of health rather than for social approval which motivates an increasingly large number of middle-aged and older men to "go on a diet." The actuarial data, although obviously suffering from a variety of deficiencies such as the failure to differentiate between different kinds of "overweight," are unanimous in emphasizing the detrimental effects of obesity. The increased incidence of degenerative diseases such as hypertension, coronary disease, and diabetes among the overweight has been firmly established. With a good deal of justification caloric overnutrition has been termed the most prevalent American nutritional disease.

The fact that increased morbidity means a lowered work efficiency and that increased mortality deprives society of individuals with valuable accumulated experience has been realized by an increasing number of large corporations particularly concerned, very naturally, about the health of their senior personnel. It is not surprising, therefore, that a reducing regimen currently attracting a good deal of attention has been proposed by a member of the medical division of the DuPont Company. A. W. Pennington approached the problem as an industrial physician. He was concerned principally with the practical problem of obesity in industry and with its solution.

Few will quarrel with his statement (*Indust. Med. Surg.* **18,** *259 (1949)*) that appetite-reducing drugs, hormonal medications, or prescriptions of strenuous exercise have not worked satisfactorily in practice. He also states that the low caloric diets, which are generally considered as the only promising way to reduce body weight, frequently produce undesirable concomitants of lassitude, weakness, reduction in the individual's energy expenditure, and decreased productivity. The psychologic complications (hunger, discouragement due to slow progress) connected with either the heroic or the more moderate restriction of calorie intake may be considerable. The increasing search for new methods of weight reduction is a cogent proof of the fact that the technics tried in the past were not always satisfactory.

Dr. Pennington's diet is definitely contrary to accepted medical practice and definitely counter to established physiologic principles of nutrition. It promises what has been considered, on good grounds, as impossible.

The principal point is the use of fat in the diet in large amounts. Pennington (*Delaware State Med. J.* **23,** *79 (1951)*) prescribes "nine ounces of lean meat and three ounces of fat, cooked weight, at each of the three meals of the day, with the patient at liberty to take more, if he chooses, in that proportion—three parts of lean to one of fat." If all of the prescribed food were consumed by the patient, he would secure approximately 2500 calories from the 27 ounces of lean meat (depending on the water and fat content), and from the 9 ounces of fat another 2000 calories. A diet providing in the range of 4000 calories per day is a strange reducing measure, indeed. In an earlier paper (*Indust. Med. Surg., loc. cit.*) the value of 3000 calories is given as the basic diet. Pennington stresses the 3:1 (by weight) ratio of protein and fat. For patients who desired to eat more than the basic diet, the total caloric intake was unrestricted. Actually, the details concerning the diet are scanty. Salt was restricted, but it was not specified how rigorous the salt restriction was; it appears to have been eliminated altogether. No limitation was placed on the

intake of fluids except that alcohol (as well as bread and sugar) was not allowed.

The principle of a low carbohydrate and high protein content of reducing diets is accepted generally. E. J. Stieglitz ("A.M.A. Handbook of Nutrition," *Chapt. 16, 2d edition, Blakiston, New York (1951)*) recommends that the major restriction in calories should be in carbohydrates (and fats), while protein intake should not be limited and often may be increased to advantage. In providing large amounts of fat in the diet and a large total caloric intake Pennington's recommendations are directly opposite to the fundamentals of energy metabolism as established and retested ad infinitum since the beginnings of nutritional science.

Pennington provides arguments to make the program sound scientifically acceptable, but no data to support the arguments. There is a table (*Indust. Med. Surg.* **18,** *260 (1949)*) indicating that 20 obese individuals of unspecified age, sex, or height lost an average of 22 pounds of body weight (range 9 to 54 pounds) in an average time of three and one-half months (range one to six months). Their actual calorie intake was not reported. This is a crucial omission in dealing with a problem in which calorie intake (and output) plays an important role.

There is a good possibility that the subject never consumes all of the fat, since one of the large factors in food waste is the fat which is left on the plate (C. M. McCay *et al., J. Am. Dietet. Assn.* **21,** *88 (1945)*). The loss of fat is likely to be even greater where there is nothing but the meat with which to absorb it. One of the simplest observations that should be made in a more objective study of the Pennington reducing regimen is the determination of the amount of fat left on the plate and in the cooking utensils of the patient who is undergoing this reduction regimen.

In a report entitled "Obesity and 'the surface area law' " (*Indust. Med. Surg.* **20,** *69 (1951)*) Pennington states that "though space forbids a fuller analysis, it may be mentioned that the results of a wide survey of biochemical developments in intermediary metabolism during the past decade point to a defect in the enzymatic oxidation of pyruvate as the essential metabolic defect in 'simple obesity.'" To substantiate that conclusion, he refers to Z. Godlowski ("Carbohydrate metabolism in obesity," *Edinburgh Med. J.* **53,** *574 (1946)*), and some of Pennington's work which is "in press." Godlowski presented no evidence to show that "a defect in pyruvate metabolism" occurs in obesity.

Another report from Pennington (*Delaware State Med. J., loc. cit.*) presents many theoretical considerations with which he attempts to support his thesis that "the tissues have a limited capacity to burn carbohydrate and that when that substance is presented to the tissues in excess of that capacity it will be broken down as far as the pyruvic acid state and then converted to fatty acid; while fat, in the form of fatty acids and ketones, will burn in an unlimited amount, as long as it is presented to the tissues." The fundamental consideration that is omitted from his presentation is how the body can be stimulated to burn enough calories to permit an obese individual to lose weight on the high caloric content of the diet he recommends for reducing. In terms of modern, or ancient, physiology this is an impossible assumption.

The facts have not been provided to permit acceptance of the concept that a person can reduce on a diet providing 3000 calories or more. In view of this basic deficiency, the theoretic matters may be discussed with brevity. In the past the elimination of fat from reduction diets was motivated by the high caloric content of fat within its small bulk. It may be that the practice went too far in this direction and tended to disregard the noncaloric role of fat in the diet, principally the satiety value.

The two papers concerned with obesity and the surface area law and the calorie re-

quirements of the obese (*Indust. Med. Surg.* **20,** *69, 267 (1951)*) provide neither data supporting the "eat-all-you-want-and-reduce" principle nor convincing theoretic argument for abandoning the ridiculed "calorie count" and for the acceptance of a high fat diet for the treatment of the obese.

Obesity is an important nutritional problem. Use of higher fat content in the reducing diet requires controlled experimentation in order to confirm the reducing efficacy of the "high-fat" diet and to evaluate the specific features of the diet which singly or in combination could be responsible for the weight reduction. In principle, it is the *fat* reduction, not *weight* reduction, which is desired. Proper experimentation must be carried out in terms of body compartments, not of gross weight. The role of the absence of salt in the diet also deserves a greater emphasis and a more critical evaluation than it has received. Above all, an accurate accounting of caloric intake must be undertaken. One does not question the weight losses reported by Dr. Pennington, but one does question the reported caloric intakes.

ENLARGED HOCK DISORDER IN TURKEYS

An enlarged hock condition in turkeys is responsible for substantial economic losses by turkey growers. It resembles the condition known as perosis in chicks and has recently been studied rather extensively by M. L. Scott. Since in many cases the tendon of achilles does not slip from its condyle, as it usually does in perosis, this abnormality in turkeys has been more properly considered as an enlarged hock (Scott, *J. Nutrition* **40,** *611 (1950)*).

Scott noted that the enlarged hock condition could not be prevented unless the diet contained at least 850 mg. of choline per pound. The allowance of choline for turkeys recommended by the National Research Council is 920 mg. per pound of feed; this recommendation is based largely on the earlier work of R. J. Evans (*Poultry Sci.* **22,** *266 (1943)*). It was further noted that choline was not completely effective, even at twice the recommended level in the diet, unless the diet contained betaine, or relatively high levels of glycine (2.25 per cent of the diet).

Recently Scott has reported further studies on the enlarged hock condition. Since glycine is a precursor of creatine, the importance of the latter as a preventive agent was investigated (M. L. Scott, *Poultry Sci.* **30,** *839 (1951)*). The basal diet used in the study had the following percentage composition: cornmeal, 39.5; pulverized oats, 10.0; ground wheat, 5.0; soybean meal (44 per cent protein), 20.7; crude casein, 18; dicalcium phosphate, 3.5; limestone, 1.5; fish liver oil (2000 I.U. of vitamin A and 400 I.U. of vitamin D), 1.0; salt, 0.8; and manganese sulfate, 0.04. Each pound of the above basal diet was fortified with the following vitamins (in milligrams): riboflavin, 2; calcium pantothenate, 2; pyridoxine hydrochloride, 2; vitamin K, 2; thiamine hydrochloride, 0.5; niacin, 10; choline chloride, 500; biotin, 0.025; and vitamin B_{12}, 0.010.

Ten to 15 poults of mixed sex were used for each treatment. In the first trial it was found that when the basal diet contained 0.4 or 0.6 per cent of creatine as a supplement, no enlarged hocks were present at the end of four weeks. Poults on the basal diet had a 67 per cent incidence of the condition at four weeks. Supplements of fish meal and meat scrap (5 per cent of each) gave almost complete protection.

Since the above results suggested that the enlarged hock condition involved a disturbed creatine metabolism, the second trial was conducted to determine the rate of excretion of ingested creatine. Two lots of poults were fed the basal diet supplemented

with 0.2 per cent of creatine. At the end of each week excreta were collected for a twenty-four-hour period for creatine analysis. The results indicated an excellent correlation between the incidence of enlarged hocks and creatine excretion. At the end of the first week, when 23 per cent of the poults had enlarged hocks, 40 per cent of the ingested creatine was excreted. This excretion rose to 59 per cent at the end of the second week when 46 per cent of the poults had enlarged hocks.

A study was then initiated to determine the effect of various substances that might influence creatine retention. The following were tried on the basal diet supplemented with 0.2 per cent of creatine: folacin, 1 mg. per pound of feed; alpha-tocopherol acetate, 50 mg. per pound of feed; inositol, 0.2 per cent of the diet; and para-aminobenzoic acid, 0.2 per cent of the diet. These all reduced the incidence of enlarged hocks and also improved creatine retention. Inositol and para-aminobenzoic acid were most effective. However, the protective influence of either inositol or para-aminobenzoic acid was almost completely eliminated if the diet was supplemented with aureomycin (2 mg. per 100 g. of diet). These results were interpreted as indicative that inositol and para-aminobenzoic acid favored the synthesis (by microorganisms) in the intestinal tract of a factor essential for the prevention of enlarged hocks, and that aureomycin acted by inhibiting these microorganisms.

The creatine content of the leg muscles of poults under various dietary conditions was also investigated. On the basal diet alone, muscle creatine averaged 455 mg. per 100 g. of muscle, contrasted with an average of 540 mg. per 100 g. of muscle when the basal diet was supplemented with 1 per cent of glycine and 0.2 per cent of betaine.

The disturbance in creatine metabolism associated with the enlarged hocks suggested to Scott that this condition might be synonymous with the muscle dystrophy in mammals that is preventable with vitamin E. Tocopherols and tocopherol-inositol complexes were, however, of no striking benefit as curative or preventive agents in the enlarged hock condition.

Various crude materials were tested for their ability to prevent the enlarged hock condition, and reported on in another paper (M. L. Scott, *Poultry Sci.* **30,** *846 (1951)*). Dried brewers' yeast was found to be an excellent preventive. Grass juice appeared to be a fair source of the preventive agent. Contrary to its action when fed with inositol and para-aminobenzoic acid, aureomycin appeared to exhibit preventive activity when fed with any one of the following: dried distillers' solubles, dried skim milk, dried whey, and grass juice.

It was noted that fish liver oils in the diet increased the incidence of enlarged hock condition markedly. When the 1 per cent of fish liver oil in the basal diet was replaced with a stabilized vitamin A preparation and irradiated animal sterols, the enlarged hock condition was completely prevented. The addition of 4 per cent of fish liver oil to the above diet resulted in a 70 per cent incidence of the enlarged hock condition and reduced the muscle creatine level from 450 mg. per 100 g. to 310 mg. per 100 g. of muscle.

The striking action of brewers' yeast in preventing the enlarged hock condition and the negative results with tocopherol alone call to mind the observations by S. Morgulis and H. C. Spencer (*J. Nutrition* **11,** *573 (1936)*), who presented evidence that a water-soluble factor was required in addition to tocopherols for the prevention of nutritional muscular dystrophy.

The results of Scott with turkey poults indicate that a disturbed creatine metabolism is present in the enlarged hock condition. Whether or not a new dietary factor is involved, however, is purely speculation. A comparison of the results obtained on the basal diet in experiments reported in the two recent papers indicates that the inci-

dence of enlarged hocks may vary from 40 per cent to 70 per cent. With such variations between experiments the validity of some of the conclusions must be questioned.

PYRIDOXAL PHOSPHATE AND CYSTINE SYNTHESIS

It is now established that cystine is not synthesized in the body from catabolic nitrogenous sources, but rather that it receives its sulfur from methionine by way of homocysteine, and the remainder of the carbon structure from serine. The intermediate in this transformation has been isolated (F. Binkley, *J. Biol. Chem.* **191,** *531 (1951)*). The enzyme preparation employed was made from rat livers; they were homogenized in 0.2 molar potassium chloride and 0.01 molar sodium citrate, centrifuged and the residue discarded. By fractionation with cold ethyl alcohol two enzyme systems were obtained, one apparently capable of effecting a synthesis of the intermediate from homocysteine and serine, and the other producing cysteine as one of the reaction products. Having prepared the synthesizing enzyme, it was possible to assay the cleavage enzyme, and vice versa. The intermediate produced by the reaction of the synthesizing enzyme upon a mixture of DL-homocysteine and DL-serine was isolated from the digest and shown to be L-cystathionine.

Further studies (F. Binkley, G. M. Christensen, and W. N. Jensen, *J. Biol. Chem.* **194,** *109 (1952)*) on the properties of the enzyme mixtures have shown that solutions of the enzymes can be inactivated by aging in a refrigerator or by dialysis against distilled water. It thus was possible to study the coenzymes involved in the overall synthesis of cysteine, as well as in the separate synthesizing and decomposing phases of the reaction. Pyridoxal phosphate added to the aged enzyme solutions increased both the synthetic and the cleavage activity as measured by the concentrations of cystathionine and cysteine respectively. Even freshly prepared enzyme preparations show some activation by pyridoxal phosphate. When djenkolic acid, L-cystathionine or L-allocystathionine were used as substrates, the stimulating action of pyridoxal phosphate on the cleavage enzyme was observed.

In order to demonstrate the coenzyme action of pyridoxine in another way, enzyme preparations were made from livers of rats subjected to various dietary adjustments: one group of rats was given 4-aminopteroylglutamic acid, another group was deprived of folic acid, and still another group was given a pyridoxine-deficient diet. In comparison to the results obtained with the liver enzymes of the control rats, only the livers of the pyridoxine-deficient group yielded preparations of low potency with respect to synthesizing and cleavage enzymes. Furthermore, the addition of pyridoxal phosphate to both synthesizing and cleavage enzymes produced a striking response in activity, of greater magnitude than observed when the vitamin was added to preparations from the control animals. The foregoing is convincing experimental evidence that pyridoxal phosphate is the co-factor in the enzyme systems involved in the transfer of sulfur in the course of the biosynthesis of cysteine.

PERNICIOUS ANEMIA IN CHILDHOOD

Although pernicious anemia is usually a disease of adult life with a peak incidence occurring in the sixth decade, it may occur during childhood. The essential defect in pernicious anemia is absence of the intrinsic factor of Castle from the gastric secretions,

with failure to absorb the extrinsic factor present in foods, now considered to be identical with vitamin B_{12}. This defect is associated with histamine-refractory achlorhydria, macrocytic anemia, arrest of maturation of the bone marrow at the megaloblastic level, a response to specific treatment, and the necessity of continued therapy to prevent relapses. Other common findings are combined system disease of the brain and spinal cord, gastrointestinal lesions, and premature graying of the hair. The number of cases of pernicious anemia fulfilling these criteria and occurring in children is extremely small, only 7 cases being recognized by E. H. Reisner, J. A. Wolfe, R. J. McKay, and E. F. Doyle (*Pediatrics* **8,** *88 (1951)*). B. Benjamin (*Am. J. Dis. Child.* **75,** *143 (1948)*) reported a case in which absence of the intrinsic factor was suggested by the Reimann technic (the failure of the gastric juice to potentiate the effect of oral liver in a patient with proven pernicious anemia in relapse), despite the inconstant presence of free hydrochloric acid in the gastric secretions. This observation suggested to Reisner *et al.* that persistent achlorhydria might not be a necessary diagnostic feature of pernicious anemia in childhood.

Four cases, two pairs of siblings, in which such a diagnosis was established have been reported by these workers. In 3 of these, free hydrochloric acid was found, although achlorhydria developed in one of them after seven years of observation. The diagnosis was confirmed by the absence of any other known cause for anemia and the demonstration that small oral doses of vitamin B_{12} were ineffective without the addition of normal gastric juice. A Reimann test was negative in one of these patients.

Twelve other cases which were felt to represent true pernicious anemia in childhood were found in the literature when the possibility of a defective intrinsic factor production in the presence of free hydrochloric acid was considered. Certain interesting observations were made on this group of 16 cases. Consanguinity or a familial history of the disease was found in 8 of the 14 families represented. The onset could frequently be traced back to infancy. Free hydrochloric acid was present in the stomach in 8 of these cases. There was evidence of combined system disease in 5, with very severe involvement which responded to specific therapy in a child of 3 years. Glossitis, gastrointestinal lesions, and even graying of the hair have been reported.

These studies have suggested that pernicious anemia may occur in children more frequently than has been supposed and that histamine-refractory achlorhydria may not be essential to the diagnosis if evidence for the absence of intrinsic factor is obtained. Benjamin (*loc. cit.*) has suggested that the defect in the stomach may have been a congenital one in his case, the anemia developing when transplacental stores of the maturation factor were exhausted. The absence of spontaneous remissions in several children in this group would also suggest that the defect might be complete from birth rather than developed later as a "degenerative" process. Pernicious anemia should be considered in the differential diagnosis of all relapsing anemias of childhood.

COMPLEX COMPOUNDS OF FLUORINE IN CARIES CONTROL

The discovery that fluoride is the etiologic factor in the development of dental fluorosis was followed by the repeated demonstration that this ion likewise exerts a protective action against dental caries. As a result, many communities are adding sodium fluoride to the communal water supply as a public health measure, thus taking advantage of the anticariogenic action of fluoride. Inasmuch as the entire water supply of a given community is ordinarily fluorinated the item of cost is significant; in

June 1951, for instance, the cost per pound of available fluorine derived from sodium fluoride was roughly three times that from sodium silicofluoride. It becomes important, therefore, to have at hand fundamental data with respect to the physiologic activity in the organism of simple as well as complex compounds of fluorine, with a view to their possible more economical use in caries control. Differences may exist between compounds regarding the availability of the contained fluorine due to absorption which, in turn, is conditioned in part by solubility. Furthermore, there may be unpredictable differences in metabolism and deposition of the fluorine in tissues, which would affect the value of these compounds as anticariogenic agents.

A recent study (I. Zipkin and F. J. McClure, *Pub. Health Reports* **66,** *1523 (1951)*) was carried out on laboratory rats using the following criteria for evaluating the efficacy of various compounds of fluorine when administered both by intraperitoneal injection and by mouth in the drinking water: (1) deposition of fluorine in the bones and teeth, (2) development of typical incisor striations, and (3) ability to inhibit experimental rat caries. The compounds which were administered were Na_2SiF_6, Na_2PO_3F, KPF_6, NaF and CF_3COONa, all being given in amounts of 50 parts per million (p.p.m.) in the drinking water and all (except CF_3COONa) being injected in amounts of 3.5 mg. per week in a concentration of 500 p.p.m. The basal cariogenic ration itself contained 1.3 p.p.m. fluorine. The animals were given the experimental diet at 21 to 27 days of age. After ninety-one days the teeth were examined for caries, and the tissues (femurs, mandibles, dentine, and enamel of molars and incisors) were dried, defatted, ground, ashed and analyzed for fluorine. It is pointed out that, in calculating the administered fluorine, the purity of the compound, both incidental and as published, must be accounted for. Thus the KPF_3, Na_2PO_3F and CF_3COONa contained free fluorine which was removed before administration to the animals. Furthermore, drastic fusion technics were necessary before the fluorine in CF_3COONa could be secured in titratable form.

Using the Cox system for the diagnosis of caries (G. J. Cox, M. L. Dodds, S. F. Dixon, and M. C. Matuschak, *J. Dent. Res.* **18,** *469 (1939)*), Zipkin and McClure observed that none of the fluorine compounds employed produced a reduction in the incidence of caries, when given intraperitoneally. That fluorine did reach the teeth was demonstrated by the increase in fluorine content of the dentine and enamel, but it is suggested that the fluorine did not reach the enamel surface in time or in sufficient amount to exert the cariostatic effect produced by oral administration. The failure of parenterally administered fluoride to prevent caries in the rat has been noted before (F. A. Arnold and F. J. McClure, *Ibid.* **20,** *457 (1941)*). Neither CF_3COONa nor KPF_6 gave evidence of caries inhibition when given by mouth. That this may be generally true for KPF_6 is indicated by the failure of this compound to prevent caries in hamsters (J. W. Hein *et al.*, *Ibid.* **30,** *466 (1951)*). Greatly increased content of fluorine in the dentine and enamel, and reduction in caries was observed in those rats receiving NaF, Na_2SiF_6 and Na_2PO_3F by mouth.

With respect to the localization of fluorine in the nondental osseous tissues studied, the complex fluorine compounds KPF_6 and CF_3COONa were different from the other fluorides employed; the physiologic availability of the fluorine in these two compounds appears to be very low. There appeared to be no difference with respect to the deposition of fluorine in the skeletal tissues between oral and injected NaF, Na_2SiF_6 or Na_2PO_3F. From the data on caries reduction and striations in the enamel, and also with respect to the increase in the fluorine in osseous tissue, it appears that Na_2PO_3F is metabolized in a manner similar to NaF and Na_2SiF_6, namely, with the release of fluoride ion. The conclusion seems

warranted, on the basis of these recent biologic data, that the use of Na_2SiF_6 in the fluoridization of domestic water supplies is justified.

BILE SUBSTITUTES AND STEATORRHEA IN DOGS

The absorption of fat from the intestine is well known to be dependent in part upon the presence of bile. In instances of obstruction or diversion of bile flow, or in the presence of liver damage the problem of steatorrhea may be of considerable nutritional significance. The existence of such a defect in patients with hepatitis and cirrhosis has been pointed out (*Nutrition Reviews* **9,** *187 (1951)*), but therapeutic measures aimed at increasing fat absorption have not been consistently effective. In this regard, G. W. Searle and J. H. Annegers have undertaken a study of bile substitutes in dogs with external bile fistulae (*Gastroenterology* **19,** *558 (1951)*).

In the first series of experiments, the animals were given dog food mixed with lard once daily, and aqueous solutions of bile substitutes (ox bile extract, sodium taurocholate, sodium glycocholate, and oxidized cholic acid) were administered by continuous duodenal infusion at the rate of 700 cc. per twenty-four hours. Fat was fed at two levels, 36 and 51 g. per day, in separate periods of the study, and daily fecal fat excretion was measured as total lipids on the last five days of eight-day test periods for each substitute. In animals receiving 36 g. of fat without treatment, average fat excretion was 21 g. per day. In comparison with this, the administration of 6.6, 9.6, and 12.3 gram doses of ox bile extract reduced the average excretion to 9.7, 8.9, and 4.9 g. of fat respectively. Likewise, sodium taurocholate (6.9 g. daily) resulted in 2.8 g. of fecal fat excreted, and 6.6 g. of sodium glycocholate reduced fecal fat to 6.7 g. In contrast, however, the administration of 6.6 g. of oxidized cholic acid was accompanied by fat excretion of 22.2 g. daily.

Studies of animals receiving 51 g. of fat revealed excretion of 33.3 g. daily in the feces, but the administration of ox bile extract in doses similar to those used in the above experiments reduced these figures to 19.1, 10.1, and 7.4 g. respectively.

Regression analyses of the effect of ox bile in decreasing fecal fat revealed that in animals receiving 51 g. of fat a diminution of fat excretion of 2.2 g. per gram of ox bile extract occurred, while a decrease of only 1.3 g. per gram was found during the 36 g. period. Thus fat absorption per gram of ox bile extract was not constant when the dietary fat was varied. Instead it appears that the administration of this substitute allowed the absorption of approximately a constant percentage of the fat ingested.

The results obtained with sodium glycocholate and taurocholate indicate that these pure bile salt preparations increased the absorption of fat at least as effectively as the ox bile extract on the basis of grams administered. Although the taurocholate study reported here was carried out upon only one animal, previous data from the same laboratory (G. W. Searle and J. H. Annegers, *Proc. Soc. Exp. Biol. Med.* **71,** *277 (1949)*) have suggested that this substance is active in decreasing steatorrhea in bile-fistula dogs. In view of this, the fat absorption-promoting quality of bile appears to be more closely related to the bile salt content than to other constituents.

Oxidized, unconjugated forms of cholic acid salts were, by contrast, found to be ineffective in reducing steatorrhea. Trials with sodium cholate and sodium desoxycholate resulted in anorexia, vomiting and diarrhea in normal and bile-fistula dogs when the former was given, and diarrhea occurred in normal dogs when the latter was given. These results discouraged further

studies, and indicate that unconjugated salts do not facilitate fat absorption.

A second series of experiments was then undertaken on similar animals to which the same diets were given in equal feedings at twelve-hour intervals simultaneously with the oral administration of bile substitutes. On the 36 g. fat intake, 6.7 gram doses of ox bile extract yielded an average of 11.4 g. of fecal fat in comparison with the 25.7 g. in the feces when no bile substitute was administered. Similar doses of the extract given to animals receiving 51 g. fat diets yielded 15.2 g. fat excretions. From this the authors conclude that ox bile extract is an effective substitute when given orally. No statistical difference could be found between the results of oral and duodenal infusion administration. In their previous investigations Searle and Annegers (*Proc. Soc. Exp. Biol. Med., loc. cit.*) had found that bile must enter the small intestine simultaneously with food in order to be at all effective in correcting steatorrhea in such dogs. In the present experiments, therefore, they conclude that the bile and food mixtures given orally were delivered to the intestine nearly simultaneously.

Calculation of the per cent of fed fat which was absorbed in these studies with varying doses of bile substitutes revealed a consistent trend toward increased absorption with increasing doses of the substitute used. On the basis of these figures, and upon what is known quantitatively about bile flow and bile salt absorption, the authors calculate that doses in excess of 14 g. of bile salt daily would be necessary for normal fat absorption in their animals. It would thus be reasonably expected that larger doses would be necessary in human subjects with similar bile fistula defects. It is suggested by the authors that previous studies of this type have failed due to inadequate dosage of bile substitutes.

These investigations lend hope to the problem of fat absorption in patients with liver or biliary tract disease. However, similar trials with desiccated hog bile preparations in humans with hepatitis have not been encouraging (J. B. Gross, M. W. Comfort, E. E. Wollaeger, and M. H. Power, *Gastroenterology* **16,** *140 (1950)*), and apparent discrepancies have yet to be resolved in the clinical use of these agents before they should be accepted for therapy in this type of steatorrhea. There may be inherent differences in the tolerance of dogs and humans for such bile extracts, and the bile salt form and content of available preparations should be known in order to avoid untoward gastrointestinal effects which are usually consequent to the administration of bile acids and unconjugated bile salts.

OXIDATION OF L-TYROSINE

The classic method of labeling compounds for the study of their behavior in intermediary metabolism has been through the use of the phenyl group, this radical being notably difficult to oxidize in the organism. Nevertheless, both tyrosine and phenylalanine are ordinarily metabolized in a way to indicate a rupture of the benzene ring, and complete oxidation. It has been known that in the oxidation of tyrosine no ammonia is evolved, a circumstance throwing doubt upon the classic view that oxidative deamination precedes further degradation of this aromatic amino acid. Furthermore, most studies of the catabolism of tyrosine show that the ratio of atoms of oxygen utilized per mole of tyrosine is 4:1 whereas a ratio of 5:1 would be expected if the mechanism involved oxidative deamination.

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A recent study (B. Schepartz, *J. Biol. Chem.* **193,** *293 (1951)*) shows that when a liver enzyme mixture, prepared by acetone extraction of homogenized rat liver, was used the obligate step to further oxidative

degradation of tyrosine is transamination. It had been shown previously that this amino acid is susceptible to transamination (P. S. Cammarata and P. P. Cohen, *J. Biol. Chem.* **187,** *439 (1950)*). The reaction, with the foregoing enzyme preparation, proceeds when alpha-ketoglutarate is present as the amino acceptor, as judged by the disappearance of the L-tyrosine substrate, the oxygen consumed, the glutamic acid formed, and the appearance of acetoacetate as the final product. Under similar experimental conditions, L-alanine and L-aspartic acid are also deaminized in the presence of alpha-ketoglutarate as judged by the amount of glutamic acid formed.

A contemporaneous study (W. E. Knox and M. LeMay-Knox, *Biochem. J.* **49,** *686 (1951)*; *Nutrition Reviews* **10,** *92 (1952)*) was carried out with a preparation made from rat livers by potassium chloride extraction, removal of mitochondria and subsequent dialysis. Again, the importance of alpha-ketoglutarate in the prerequisite transamination was demonstrated, but with this more refined enzyme preparation, catalytic traces of ascorbic acid were also required for the complete oxidation. It was found by ammonium sulfate precipitation of the fresh enzyme, followed by dialysis, that the coenzyme of the transaminase was apparently removed, thus reducing the rate of amino-transfer and limiting the rate of the over-all oxidation of the tyrosine. The full transaminase activity could be restored by adding boiled fresh enzyme or pyridoxal phosphate. Isoascorbic acid is as effective as ascorbic acid, and either one seems to be required for the continued oxidation of the product of the initial deamination, namely, para-hydroxyphenylpyruvic acid. That the relation of ascorbic acid to the oxidation of tyrosine is not directly associated with the antiscorbutic activity of the vitamin is indicated by the fact that much greater amounts of ascorbic acid are needed to prevent scurvy. Although intermediate degradation products of tyrosine were demonstrated when 0.5 g. or more per diem were given to scorbutic guinea pigs (R. R. Sealock and H. E. Silberstein, *J. Biol. Chem.* **135,** *251 (1940)*), indications of the excretion of para-hydroxyphenylpyruvic acid were present in one study when very large doses of tyrosine were given to animals whose tissues were saturated with ascorbic acid (H. A. Painter and S. S. Zilva, *Biochem. J.* **41,** *511 (1947)*). It would appear that para-hydroxyphenylpyruvic acid, 2,5-dihydroxyphenylpyruvic acid, and homogentisic acid are intermediates in the reaction which, under these conditions, leads to the final production of acetoacetate, fumarate and carbon dioxide (R. G. Ravdin and D. I. Crandall, *J. Biol. Chem.* **189,** *137 (1951)*).

THE ANTIBACTERIAL FACTOR OF MILK

The antibacterial activity of cow's milk was noted in 1897 and has been the subject of periodic study since that time. The factor responsible for this activity has been termed "lactenin," and was shown to be a heat-labile, nondialyzable component of whey. Tryptic digestion does not destroy the factor and has permitted some purification. Lactenin was found to be variably bacteriostatic and bactericidal when tested against a number of strains of streptococci.

The antistreptococcic properties of milk have been reinvestigated recently by A. T. Wilson and H. Rosenblum (*J. Exp. Med.* **95,** *25, 39, 51 (1952)*). These authors have developed a satisfactory system for studying the activity of lactenin. All group A streptococci tested were found sensitive; however, most group B, C, D, and E strains tested proved resistant. The results with groups F, G, H, K, and L were variable. The utilization of lactenin sensitivity as a practical aid in the laboratory differentiation of group A streptococci from other groups was

considered. In testing 408 group A strains all but one proved to be inhibited by lactenin. The resistant strain was sensitive on retesting. Among 45 strains of other groups there were 2 sensitive strains and 2 strains which were partially inhibited.

The authors in investigating the resistance of lactenin to inactivating agents have discovered new properties which may have biologic and epidemiologic significance. The antistreptococcic activity of lactenin is lost in media where the oxygen tension is low. The restoration of oxygen tension resulted in a return of antistreptococcic potency. Reducing agents such as cysteine, glutathione, thioglycolic acid and BAL also were effective inhibitors of lactenin activity. Of some interest to nutritionists is the observation that thiamine in concentrations in excess of 0.38 mg. per cent inhibited the antistreptococcic action of lactenin, suggesting the possible interference of lactenin with thiamine metabolism.

The possible influence of lactenin in milk-borne epidemics of streptococcic disease is of great interest. The occurrence of bovine group A mastitis of cows is rare, but hard to understand in view of the antistreptococcic activity of milk. By collecting milk anaerobically under oil it was possible to demonstrate that there was no antibacterial action. The conclusion seems warranted that intramammary milk is in a reduced state and lactenin can no longer exert its antistreptococcic action. Lactenin, however, is activated by exposure to oxygen and accounts for the failure of growth of contaminating streptococci *after* milking. In the preparation of powdered and canned milk lactenin is destroyed, and these milk products are good media for the growth of group A streptococci. The epidemics of group A streptococci which have been spread by fresh milk are probably not the result of proliferation of the organism within the milk, but are due to the failure of complete bactericidal action in the presence of contamination with a large number of organisms.

Prospects of this substance becoming a useful antibiotic therapeutic agent are not bright. The oral or intraperitoneal administration of milk had no effect on the course of mouse streptococcic peritonitis. The apparent low potency and limited range of action even among streptococci is not promising. These studies are important, however, in helping to explain the infrequent occurrence of group A streptococcal milk-borne epidemics despite the frequent opportunities of contamination from human sources.

More information is needed as to the chemical nature of lactenin. Its relative stability should make purification and characterization possible. On the basis of the action of enzyme inhibitors the authors have concluded that lactenin action is not due to xanthine oxidase, peroxidases or lysozyme—enzymes known to be present in milk. It is not an actively induced antibody and does not require complement for its action. Lactenin from goat, cow, and human milk inhibits the same streptococci. The fact that thiamine overcomes the inhibition of lactenin has prompted the speculation that the agent might interfere with normal intramicrobial thiamine utilization.

BONE DENSITY

The "nutritional" status of the organism may be defined, in part, in terms of the physical characteristics of its tissues. In children's rickets and in osteomalacia (softening of bones in the adult), striking bony lesions are found on roentgenographic examination and serve as diagnostic signs of the disorders (N. Jolliffe, F. F. Tisdall, and P. R. Cannon, "Clinical Nutrition," *p. 100, Paul B. Hoeber, Inc., New York (1950)*). The roentgenographic changes precede the "clinical" manifestations of the disease.

There are such morphologic alterations as an enlargement of the ends and bending of the long bones and, earlier, a decrease in the density of the shadow cast by the bone on the x-ray. This rarefaction is due to failure in deposition of calcium. For a clinical diagnosis of the presence of the disease the usual roentgenographic examination is reasonably adequate, although the initial changes consisting in slight decalcifications can hardly be visualized on the roentgenogram (I. Snapper, *in* "Clinical Nutrition," *p. 251* (*loc. cit.*); *see also* E. A. Park, *Ibid., p. 449*). For a quantitative study of the process of decalcification and recalcification, and for characterizing the calcification of bones of individuals with marginal malnutrition, more precise methods must be used.

The methods proposed start from the fact that, with properly standardized technic, the density of a bone may be evaluated on the basis of the relative blackness of the image of a given bone on the x-ray film. However, there is little doubt that an impressionistic evaluation, consisting in comparing the particular film with the vague "standards" stored in the roentgenologist's memory, remains a highly subjective and scientifically unsatisfactory procedure.

The technic has been much improved by the provision of objective standards. In connection with the study of bone development such standards were provided by T. W. Todd ("Atlas of Skeletal Maturation," *C. V. Mosby, St. Louis* (*1937*)). The technic was used in studies concerned with the over-all problem of growth, such as the Adolescent Growth Study carried out by the Institute of Child Welfare at the University of California (N. Bayley, *J. Consult. Psychol.* **4,** *69* (*1940*); H. E. Jones *et al.*, "Development in Adolescence; Approaches to the Study of the Individual," *Appleton-Century, New York* (*1943*)), and the cooperative study of school children reported from the Psychological Clinic and the Department of Child Hygiene, Harvard University (R. N. Sanford *et al.*, "Physique, Personality and Scholarship," *Soc. Res. Child Develop., Serial No. 34, National Research Council Monograph* **8** (*1943*)). Roentgenograms of hand and knee, and of hand and wrist, respectively, were taken and each film was matched by inspection with Todd's standards. The results of the Brush Foundation study of human growth and development, initiated by Todd, were presented by W. W. Greulich and S. I. Pyle ("Radiographic Atlas of Skeletal Development of the Hand and Wrist," *Stanford Univ. Press, Stanford* (*1950*)).

Todd and his associates made assessments of the skeletal maturation of the subjects studied by Icie G. Macy at the Research Laboratory, Children's Fund of Michigan, in Detroit ("Nutrition and Chemical Growth in Childhood," *Vol. I, Evaluation* (*1942*); *Vol. II, Original Data, C. C. Thomas, Springfield, Ill.* (*1946*)). The assessment was based on 6 roentgenograms showing hand, elbow, shoulder, foot, knee, and hip. From the x-ray films of the hand the skeletal age of the individual child was calculated by the methods of C. D. Flory ("Osseous Development in the Hand as an Index of Skeletal Development," *Soc. Res. Child Develop., No. 3, National Research Council Monograph* **1,** (*1936*)) and H. J. Kelly ("Anatomic Age and its Relation to Stature," *Univ. Iowa Studies in Child Welfare* **12,** *No. 5* (*1937*)). In addition, use was made of the older standards compiled by B. T. Baldwin, L. M. Busby, and H. V. Garside ("Anatomic Growth of Children," *Univ. Iowa Studies in Child Welfare* **4,** *No. 1* (*1929*)). Some recent roentgenographic studies on the ossification of the bones of the hand in relation to nutritional status of children were reviewed in *Nutrition Reviews* **8,** *124* (*1950*).

An objective photometric technic for the evaluation from roentgenograms of mineral density in living human subjects and experimental animals was developed by P. B. Mack and her co-workers at the Ellen H. Richards Institute, School of Chemistry and Physics, the Pennsylvania State College. The history of the project with its roots back in 1927, the instrumental details, and

some results were presented by Mack (*in* "Nutrition in Relation to Health and Disease," *p. 30, Milbank Memorial Fund, New York (1950)*). An earlier model of the photographic densitometer was described by Mack, A. T. O'Brien, J. M. Smith, and A. W. Bauman (*Science* **89,** *467 (1939)*). Mack, W. N. Brown, and H. D. Trapp (*Am. J. Roentgenol.* **61,** *808 (1949)*) considered the theoretic aspects of quantitative evaluation of bone density, described the procedure, and discussed actual and potential applications of the technic. Mack, Trapp, and Brown brought out the essential points in Appendix I, Methods, *in* "The Biology of Human Starvation," A. Keys *et al.* (*p. 1081, Vol. II, Univ. of Minnesota Press, Minneapolis (1950)*). The technic for automatically correcting the densitometer values with reference to exposure and development conditions of the particular film was described in detail by Brown and W. B. Birtley (*Rev. Sci. Instr.* **22,** *67 (1951)*).

In the early stages of the development of the procedure for an objective evaluation of the skeletal mineral density, a stationary beam of light was passed through a selected area of the bone roentgenogram, also stationary, onto an illumination photometer (a photronic cell) identical in principal with the photographer's "exposure meter." The stationary technic, using ivory wedges as calibrating devices, was used also by I. Stein (*Am. J. Roentgenol.* **37,** *678 (1937)*). The procedure was not found satisfactory. Large errors resulted from the difficulty in locating precisely the same area in bones of different subjects or in the same subject examined at different times.

This undesirable feature was removed, at least in part, by using a stationary beam of light passed through a portion of the x-ray film moving at a standard speed over a specified distance. The height of the photometric tracing increases with the light transmitted through the corresponding point on the roentgen film. This, in turn, is a function of the amount of mineral matter, principally calcium, in the x-rayed bone. Stated more precisely, it is a function of the atomic numbers of the respective elements in the x-rayed material, with calcium responsible for the highest x-ray absorption.

The principle of tracing skeletal roentgenograms between two landmarks was retained in later modifications of the densitometric technic, designed to provide greater precision. With the original (1929) instrument, an adaptation of a photographic tracing densitometer built for scanning spectrum photographs, a poor degree of reproducibility was obtained, especially in smaller bones. However, in x-rays of the large bones of adult cadavers reasonably accurate and reproducible tracings between two points were obtained and the average mineral density of the cross section of a bone could be measured. In time, technical improvements widened the range of bones which could be studied, and at the present time the method is applicable to bones ranging in size from the mouse femur to man's skull. The density values of the bone cross section are reported in terms of the average equivalent ivory thickness, in centimeters, or as grams of bone ash per unit volume. Other ways of reporting the data also have been used (*cf. Am. J. Roentgenol.* **61,** *808 (1949)*).

The procedure of translating a densitometric tracing of the cross section of a bone, recorded on a photographic paper together with the tracing of a calibrated ivory step-wedge x-rayed on the same film, into mineral density values was very time consuming, even with Model 4 used in the early 'forties. This severely limited the amount of actual analytic work. The most recent model (Model 5), designed by W. N. Brown, greatly simplifies the labor by use of a device termed a function transformer which yields a corrected photometric trace indicating directly the equivalent ivory thickness. By means of an additional instrument, these thickness values are integrated automatically along the tracing path; the resulting value represents the ivory equivalent of the cross-sectional area of the portion of the

bone traced. Taking into account the actual bone cross-sectional areas, estimated from exterior measurements of the bone and of the cavity determined from the x-rays taken at right angles to each other, and the density of ivory, one arrives at the "ivory density" of the bone (grams of equivalent ivory per cubic centimeter of bone). Using a conversion factor, the bone density may be given in grams of bone ash per unit volume.

Materials other than ivory may be used for making the standard wedges, provided the x-ray penetration of the material is not greatly different from bone. An alloy which is very similar to that of bone in x-ray absorption has been devised at the Ellen H. Richards Institute, and is now being used experimentally as wedge material (*personal communication*). This should simplify the expression of the values in terms of bone density (grams per cubic centimeter of bone) and would make possible omitting ivory as an intermediate factor.

Experimental checks on the reproducibility of the mineral index values, in the same bone, were reported (Mack *et al.*, *loc. cit.*, *p. 823* (*1949*)) for variations of the film-development time from two to four and one-half minutes. The values are markedly affected by this factor. When the density of the bone image in each film was corrected on the basis of the photographic trace of the ivory step-wedge x-rayed on the same film, the systematic variations were cancelled out. The residual variations occurred within about ± 0.2 units (cm.) of the mean mineral index. The significance of these random fluctuations can be established only by knowing the magnitude of differences resulting from such factors as variations in nutritional status or in age.

The reviewer is not aware of published systematic data on the reliability (repeatability) of the technic. The senior investigator (Mack, *loc. cit.*, *p. 31* (*1950*)) characterized the consistency of repeated readings in qualitative terms by stating that *highly reproducible* tracings may be obtained of bones of human infants and of experimental animals as small as young rats. Elsewhere (*loc. cit.*, *p. 817* (*1949*)) it was noted that it is possible to reproduce mineral density values, obtained for the same segment of the same bone from different films, with an accuracy of a small percentage. Standard methods of positioning the limbs or the skull at the time of taking the x-ray pictures and standardization of the position of the tracing path on the film increase importantly the accuracy of the mineral density values.

In order to determine the validity of the x-ray (photometric) evaluation of bone density, the data obtained on nine bones were compared with the mineral density found by the laboratory ashing method. The data, presented in graphic form, indicated a very close correspondence between the two sets of values; the relationship was linear and the deviations from regression were very small. Elsewhere Mack (*loc. cit.* (*1950*)) reported a coefficient of correlation of over 0.9 between mineral density found by the direct technic of ashing and the degree of mineralization estimated by the photometric technic; this value was obtained for cadaver bones of stillborn infants and of experimental animals (rats), using the earlier (fourth) model.

The results of investigations, carried on over a period of years and using the technic of estimating mineral density from x-rays, have not yet been reported systematically. In an ambitious cross-sectional study, concerned with relationship between the intake of certain major nutrients and bone density, films of over 13,000 subjects, children and adults, were obtained. Several longitudinal studies on nutrition and bone mineralization were conducted, but not written up so far. A few case studies were given by Mack (*loc. cit.* (*1950*)).

In one subject, a young male adult, member of a group of 5 conscientious objectors who served in a longitudinal study on calcium retention and bone density, intake and output of calcium in urine and feces were studied over a period of four hundred and

eighty-two days. After about four weeks on an intake equal to that recommended by the Food and Nutrition Board, National Research Council (0.8 g.), the intake was reduced to about half of that value and subsequently increased step-wise to levels fluctuating between 1.3 and 1.7 g. In the final month the calcium intake was reduced abruptly to less than 0.2 mg. daily. The output of calcium in urine and feces followed, within limits of the sensitivity of this method, the changing level of the calcium intake. Strangely enough, the subject appeared to be in a negative calcium balance for the greater part of the experiment. The mineral density of the heel bone (os calcis), determined from the x-rays, showed marked fluctuations which appeared not to be closely related to the calcium intake, output, or the difference between the two. Nevertheless, a general trend toward lower density values was discernible, and lowest mineralization values were observed during the last month. The mineral density of soft tissues near the heel bone was also determined by the same technic, but showed large and apparently random variations. It is difficult to interpret the fact that the values of mineral density of the soft tissues, given as grams of ivory per cubic centimeter of tissue, are unexpectedly high.

Another case study involved a child on a very low but not specified intake of vitamin D. The mineral density index, averaged for the two bones of the forearm (radius and ulna), increased from 0.40 to 0.50 during the first year of life and remained at that level, with small variations, for the next three years. No "normal" values for children of this age were presented. Another child in the same group who experienced many illnesses during the period of the study started from a lower level (a mineral index of around 0.30). The mineralization curve followed an upward trend, reaching a level of about 0.47 when the child was about 4 years old, but showed many ups and downs. Without a parallel graph of the clinical course, presenting in some detail a record of the pathology, the material reveals little.

Perhaps the most convincing, as the power of conviction of isolated case studies goes, is a record of a 9-year-old postrachitic girl. While a subject in good nutritional status, matched for age and sex, had about the same "density" of the soft tissues as the patient (0.274 and 0.270 g. of ivory per cubic centimeter of tissue), the heel bone density of the normal child was considerably higher (0.300 versus 0.224). Values are given also for 2 children with a congenital disease (osteogenesis imperfecta) marked by fragility of the bones due to impaired formation of bony tissues. The densities of the heel bones were very low (0.071 and 0.096), while the matched nonpathologic subjects, 9 and 4 years old, had values of 0.287 and 0.283, respectively.

The mineral content of bones and teeth in caloric deficiency was discussed in detail by Keys *et al.* (*loc. cit.* (*1950*)). Even in severe starvation the loss of skeletal weight is much smaller than the decrease in the weight of other tissues, particularly when the weight of fat-free bones is considered. Field reports from areas of famine and near-famine have reported an increased incidence of the bone disorders, and occasionally (*see e.g.* M. Lamy, M. Lamotte and S. Lamotte-Barillon, "La dénutrition," *Doin, Paris* (*1948*)), radiographic examinations of the bones of starvation victims were made and presented qualitative evidence of some decalcification. Quantitative data were obtained by H. D. Trapp, co-worker of P. B. Mack, in the Minnesota starvation experiment (Keys *et al.*, *loc. cit.*). The data were reported both as density values (integrated average density of a bone, expressed in terms of ivory thickness, in centimeters), which depend both on the size of the bone and the compactness of its structure, and as concentration values (a measure of minerals present per unit area of the bone). For the second phalange of the left finger the average density for 12 normal men was 0.478, for 32 men who lost 24 per cent of their gross body

weight, 0.499; after six and twelve weeks of nutritional rehabilitation the average density values were 0.482 and 0.454, respectively. For the heel bone the density of the normal control subjects was 1.694; in the semistarvation group measured after twenty-four weeks of semistarvation and twice in rehabilitation the values were 1.738, 1.700, and 1.747. A conclusion was drawn that under given conditions of caloric deficiency, with an average daily calcium intake close to the NRC recommendations, no general demineralization of bones took place.

The photometric technic of measuring the density of bones which reflects their mineralization and, more specifically, their calcium status has important applications in nutritional research. It may replace the tedious calcium balance technic and may provide information on the absolute status of mineralization rather than simply furnish a balance sheet of the difference between intake and output. It may provide a valuable tool for the study of changing calcium status in such conditions as pregnancy, and versatile means for the study of other factors which affect calcium metabolism, normal and abnormal.

The crucial point in demonstrating the validity of the method is to establish the correlation between the photometric mineral density data and the results of actual chemical analysis of the bones. The data presented up to date are most impressive, but the sample is small (9 bones), and further work along this line is needed.

For many years the principal effort was devoted to the improvement of instrumentation. The reported progress appears most gratifying and the stage of commercial manufacture of the equipment is within reach. Future efforts should be more in the biologic direction. After demonstrating the sensitivity of the technic to drastic deviations in calcium intake and to gross pathologic developments, the principal tasks are two: (1) establishment of adequate norms, taking into account the age factor, and (2) testing the sensitivity of the technic in experiments on diets within a more normal range of calcium intake. It is sincerely hoped that in mass nutritional studies sound sampling technics will be followed. This not only would safeguard the validity of the norms, but would reduce the amount of actual material to manageable proportions.

SERUM GLYCOPROTEINS IN EXPERIMENTAL SCURVY

The direct morphologic consequences of ascorbic acid deficiency are practically restricted to supporting tissue of mesenchymal origin (S. B. Wolbach and O. A. Bessey, *Physiol. Rev.* **22,** *233 (1942)*). Hemorrhages, weakness, and symptoms and signs related to articular and skeletal lesions are common features of both experimental and clinical scurvy. It is believed that ascorbic acid is not necessary for the survival and multiplication of the cells concerned with matrix formation, but is specifically concerned with the elaboration and/or maintenance of the intercellular matrix of mesenchymal tissues. Histochemical studies by B. Sylvén (*Acta chir. Scandinav.* **86,** *Suppl. 66 (1941)*) indicated that mucopolysaccharide sulfuric acid esters appeared early in the intercellular substance of healing wounds in normal animals. These substances were identified by their metachromatic appearance when stained with toluidine blue. Reduction in the metachromasia of articular cartilage stained with toluidine blue in scorbutic animals was demonstrated by A. Meyer (*Z. f. Vitaminforsch.* **14,** *332 (1943–44)*). J. R. Penney and B. M. Balfour (*J. Path. Bact.* **61,** *171 (1949)*) confirmed the production of acid mucopolysaccharides in the early stages of wound healing in normal guinea pigs and observed a decrease in the apparent production of these substance in the wounds of

guinea pigs depleted of vitamin C. Following the intramuscular injection of ascorbic acid, there was a rapid appearance of the mucopolysaccharides in the wounds of depleted animals.

H. Bunting and R. F. White (*Arch. Path.* **49,** *590 (1950)*) have also observed the presence of material in healing wounds which stains metachromatically with toluidine blue. When the tissue sections were treated with hyaluronidase of testicular origin prior to staining with toluidine blue, no metachromasia resulted. No such effect was observed when the tissues were treated with hyaluronidase of streptococcic origin. The testicular enzyme affects mucopolysaccharides containing sulfate as well as those without, while the streptococcic enzyme affects the latter group only. Bunting and White conclude that the substance responsible for the toluidine blue metachromatic staining is probably a sulfate-containing mucopolysaccharide. The wounds of animals in a state of relative, but not complete, scurvy had larger amounts of the metachromatic material than those of comparable controls. This is in contrast to the marked decrease in mucopolysaccharides in the wounds of completely scorbutic animals observed by Penney and Balfour, but perhaps in keeping with their observation of the rapid appearance of abundant mucopolysaccharides following the administration of ascorbic acid.

The site (or sites) of the production of the acid mucopolysaccharides in the healing wounds has not been established. Sylvén had suggested that the metachromatic substance was derived from mast cells, but Bunting and White found no correlation between the abundance or scarcity of mast cells and the degree of metachromasia of the tissue. They suggest that perhaps the fibroblasts (and other mesenchymal cells) are capable of elaborating the mucopolysaccharides.

Penney and Balfour observed the formation of very fine argyrophil fibers following the initial production of the mucopolysaccharides in healing wounds. There was a progressive thickening of these fibers and a concomitant change in the distribution of the mucopolysaccharides toward a fibrous form corresponding to the argyrophil fibers. They suggest that the fiber protein is closely connected with the mucopolysaccharide, either in physical combination or as a complex.

Depolymerization of the ground substance of connective tissue in a variety of disorders, including scurvy, has been suggested by I. Gersh and H. R. Catchpole (*Am. J. Anat.* **85,** *457 (1949)*) on the basis of various histochemical studies. The increased metachromasia observed in the healing wounds of partially scorbutic animals may be due to an increased reactivity to toluidine blue rather than to an increased amount of the metachromatic material on the basis of depolymerization of the ground substance (C. L. Pirani, R. C. Stepto, and K. Sutherland, *J. Exp. Med.* **93,** *217 (1951)*). Gersh and Catchpole have also suggested that depolymerization of the glycoprotein constituents of the ground substance would lead to an increase of their water solubility and to an increase of their concentration in blood serum. Accordingly, Pirani and Catchpole (*Arch. Path.* **51,** *597 (1951)*) have studied the level of serum glycoproteins in experimental scurvy.

Four groups of young adult male guinea pigs were studied: (1) controls, receiving a supplement of 2.5 mg. vitamin C per day orally, or adequate greens (lettuce); (2) "chronic scurvy," receiving 0.2 mg. vitamin C per day; (3) "acute scurvy," receiving no vitamin C; (4) "rehabilitation," a small group of animals rendered scorbutic and then fed a diet adequately fortified with vitamin C for seven days. Guinea pigs pair-fed with animals in groups 2 and 3 were available as additional controls. The serum specimens were analyzed for glycoproteins by the methods of R. J. Winzler, A. W. Devor, J. W. Mehl, and I. M. Smyth (*J. Clin. Invest.* **27,** *609 (1948)*): (1) sugar was

determined by the orcinol reagent against a standard of galactose-mannose; (2) tyrosine was determined by the Folin-Ciocalteu reagent.

The serum glycoprotein concentrations of guinea pigs with acute or chronic scurvy were significantly higher than in normal guinea pigs. No significant difference was observed between the glycoprotein values of the two scorbutic groups, nor did there appear to be a close correspondence between the serum glycoprotein levels and the estimated severity of the disease as judged by enlargement of the joints, costochondral beading, and extent of hemorrhage in these same areas. The possible influence of inanition was partially controlled by the use of control animals pair-fed with the deficient animals, although the animals receiving ascorbic acid lost less weight than the scorbutic animals. Normal serum glycoprotein concentrations were found in the pair-fed controls. In the vitamin-C treated group of scorbutic animals there was clinical improvement in some, but not all, while the serum glycoprotein concentrations fell somewhat. However, the levels of glycoproteins of this group were still significantly higher than the levels of normals. At autopsy, the lesions of scurvy, although less prominent than in the scorbutic animals which received no vitamin C therapy, were still obvious.

Pirani and Catchpole point out that earlier work by Catchpole (*Proc. Soc. Exp. Biol. Med.* **75,** *221 (1950)*) on the depolymerizing reaction indicated that altered connective tissue might be the source of the increased serum mucoproteins observed in animals with certain types of trauma, infections, and tumors. They interpret the findings of increased serum glycoprotein concentrations in scorbutic animals as lending support to the concept of a depolymerized ground substance as a feature of scurvy. Pirani and Catchpole suggest that the elevated serum glycoproteins could result either from the failure of glycoproteins to be polymerized in the absence of ascorbic acid, or from the depolymerization of glycoproteins of normally formed ground substance. They are of the opinion that both factors are involved, though no conclusive evidence is available on which to base a definite conclusion. They raise the question whether there is actually a failure of formation of extracellular material in scurvy as has been assumed. They speculate that perhaps extracellular material is produced in scurvy, but because of depolymerization it enters the blood stream and is not deposited about connective tissue cells as in normal subjects. Pirani and Catchpole feel that complete cessation of the production of ground substance probably occurs only in the very advanced scorbutic state, while depolymerization of the ground substance develops earlier in the disease. This would be in keeping with the histologic and histochemical observations of earlier investigators.

The study of Pirani and Catchpole is a stimulating one. There appears to be a relationship among vitamin C, depolymerization of connective tissue ground substance, and serum glycoproteins. However, as they point out, depolymerization and elevation of serum glycoproteins may occur in a variety of conditions and in the presence of a presumably adequate intake or synthesis of vitamin C. In this regard, depolymerization has been observed in certain tumor-bearing mice, a species which does not ordinarily require an outside source of ascorbic acid (H. R. Catchpole, *Proc. Soc. Exp. Biol. Med., loc. cit.*). Pirani and Catchpole suggest, however, that systemic or local ascorbic acid deficiency should be considered as a possible factor in instances other than obvious scurvy involving connective tissue change. Further work in this field with more precise characterization of the compounds and conditions involved is indicated.

NOTES

Niacin and Penicillin Dermatitis

G. E. Morris (*New Engl. J. Med.* **244,** *758 (1951)*) has observed 20 cases of genital and rectal dermatitis which followed the administration of penicillin. Redness, vesicles, papules, moisture and scaling of the parts were seen. It was also noticed that diarrhea and stomatitis occurred in 5 and 4 patients, respectively. Dermatitis of the exposed areas was not seen. One hundred mg. of niacin was given three times daily by mouth to these patients, as a therapeutic measure. The author states that healing was usually complete within twelve days after institution of this therapy. He further postulates that penicillin may have interfered with the conversion in the body of tryptophan to niacin. To establish this interpretation as the correct one will require data not at present in hand. Moreover, the slow recovery sometimes observed casts some doubt upon niacin deficiency as the cause of the phenomenon. It might be wondered, for example, how rapid recovery is after penicillin withdrawal alone.

Wartime Starvation

Since the end of the last world war a number of reports of studies on wartime starvation have appeared. Some of these have been reviewed in this journal. The following references on starvation in Europe may be of interest: in western Europe, "Enseignments de la guerre 1939–1945 dans le domaine de la nutrition," edited by E. J. Bigwood, *Desoer, Liége (1947)*); France, "Etude des troubles causés par la dénutrition dans un asile d'aliénés," M. Bachet, *Arnette, Paris (1943)*); Belgium, "Les états de carence en Belgique pendant l'occupation Allemande 1940–1944," edited by L. Brull, *Soledi, Liége (1945)*), and "La dénutrition de guerre: Etude clinique, anatomo-pathologique et thérapeutique," E. F. Simonart, *Acta Med. Belg. (1948)*; western Holland, "Medical Experiences in the Netherlands During the Occupation, 1940–1945," (in Dutch) edited by I. Boerema, *Wolters, Groningen (1947)*, and "Malnutrition and Starvation in Western Netherlands, September 1944–July 1945," edited by G. C. E. Burger, J. C. Drummond, and H. R. Sandstead. In two parts. *General State Printing Office, The Hague (1948)*.

The reports have included experience with the victims of German concentration camps, "Hungerkrankheit, Hungerödem, Hungertuberkulose," A. Hottinger *et al., B. Schwalbe, Basel (1948)*, and "La dénutrition: Clinique, biologie, thérapeutique," M. Lamy, M. Lamotte, and S. Lamotte-Barrillon, *Doin, Paris (1948)*; starvation in the Warsaw Ghetto, "Maladie de famine: Recherches cliniques sur la famine exécutées dans le Ghetto de Varsovie en 1942," edited by E. Apfelbaum, *Am. Joint Distribution Committee, Warsaw (1946)*, and the besieged Leningrad (*cf.* bibliographic entries under Leningrad in "The Biology of Human Starvation," A. Keys *et al., Univ. of Minnesota Press, Minneapolis (1950)*); in Greece, T. Doxiadès, *Rev. méd. de la suisse rom.* **70,** *224 (1950)*; in western Germany, "Studies of Undernutrition, Wuppertal 1946–9," R. A. McCance *et al., Med. Res. Council, London, Special Report Series* **275** *(1951)*.

Recent Nutrition Books

Nutrition and Climatic Stress. H. H. Mitchell and Marjorie Edman. Charles C. Thomas, Springfield, Ill., 1951. Pp. 234.

Food, the Growth of Policy. Volume 1 in the series "History of the Second World War." R. J. Hammond. Pp. 404. $5.75. Copies may be purchased through the British Information Services, 30 Rockefeller Plaza, New York 20, N. Y.

Clinical Nutrition Briefs

"It is thus apparent that conclusions as to the necessity for pure choline or methionine in addition to a nutritious diet in the treatment of cirrhosis or pericarditis must be held in abeyance. In the majority of patients with hepatitis, however, lipotropic agents appear to be without benefit." (*Nutrition Reviews* **10,** *12 (1952)*, Lipotropic Factors and Phospholipid Turnover in Hepatitis and Adhesive Pericarditis.)

"There is a close relationship between chronic disease and the aging of tissues, and nutrition is of great importance to both. 'Aging need not be synonymous with degeneration,' and adequate nutrition can be expected to prove the truth of this statement." (*Nutrition Reviews* **10,** *38 (1952)*, Nutrition in Chronic Disease.)

"If these studies, in spite of the defects mentioned, should reveal the recommended therapy to be a truly effective method of preventing thromboembolic disease, the mechanism of action of vitamin E still remains unclear." (*Nutrition Reviews* **10,** *46 (1952)*, Alpha-Tocopherol and Thromboembolic Disease.)

"The findings described pose a question concerning the validity of attempts to assess the nutritional status of an individual relative to riboflavin by determinations of rates of urinary excretion, since this is shown to relate directly to nitrogen balance in or just preceding the period of study. These investigations demonstrate the interdependence of protein and riboflavin metabolism in man. Also, they indirectly describe the flavoproteins as one component of a larger pool of quite labile body proteins." (*Nutrition Reviews* **10,** *58 (1952)*, Riboflavin Excretion and Nitrogen Balance.)

"The data support the conclusion that the cariostatic effect of fluoride is exerted in adults as well as in children who are native to areas whose potable water contains this ion." (*Nutrition Reviews* **10,** 74 *(1952)*, Fluoride and Caries in Adults.)

"It is estimated that there are 30,000,000 people in this country who are 10 per cent overweight and 15,000,000 who are 20 per cent overweight. It appears, from the record, that neither the present lot nor the prognosis of the obese is a happy one." (*Nutrition Reviews* **10,** *81 (1952)*, A Newer Plan of Weight Control.)

"These reports suggest that reversible fatty infiltration of the liver is a frequent result of the administration of aureomycin and terramycin in both humans and animals. However, further investigation of the nutritional and metabolic effects of aureomycin and terramycin is needed before their use over prolonged periods or in large doses can be condemned, particularly in patients in need of antibacterial therapy." (*Nutrition Reviews* **10,** *89 (1952)*, Liver Fat and Antibiotic Therapy.)